

Extraction, Denaturation and Hydrophobic Properties of Rice Flour Proteins

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ABSTRACT: Rice proteins were extracted from defatted rice flour. Turbidity measurement of supernatants revealed isoelectric points of albumin (pH 4.1), globulin (pH 4.3 and pH 7.9), and glutelin (pH 4.8), at which they were precipitated with 82.3 to 93.2% recovery efficiency. Prolamin did not aggregate and precipitate upon pH adjustment, but was precipitated by acetone. Denaturation temperatures (73.3, 78.9, and 82.2 °C) as well as enthalpy values (2.88, 3.14, and 3.79 J/g), of albumin, globulin, and glutelin were different. Prolamin did not show any thermographic denaturation peak. Heat-denaturation of globulin and glutelin resulted in progressive increases in their surface hydrophobicities. Measurement of surface hydrophobicity would be an effective parameter to evaluate rice protein denaturation.

Key words: rice proteins, extraction, denaturation, and hydrophobicity.

Introduction

THERE HAS BEEN INCREASED USE OF RICE PRODUCTS AS INGREDIENTS in gels, puddings, ice creams, and baby formulas because of their nutrition, hypoallergenicity, colorlessness, and bland taste properties (Chrastil 1992). However, there are drawbacks because of the variability in rice quality. Protein denaturation during rice drying, milling, storage, and processing can lead to different functional manifestation in foods.

Proteins and starch are the two major components of rice, with approximately 8 and 80%, respectively (Marshall and Wordsworth 1994). Rice protein is valuable because it has unique hypoallergenic properties and ranks high in nutritive quality (rich in the essential amino acid lysine) among the cereal proteins (Bean and Nishita 1985). The hypoallergenic property and the high nutritive quality could make rice protein concentrate or isolate a competitive protein ingredient in the food ingredients market. An effective extraction is essential for the commercial production of rice proteins. The residue after protein removal could be used to prepare marketable starch. Rice starch is widely used in cosmetics and medicine (Chrastil 1992), but the use of rice protein in food systems is now limited due to its unavailability and unknown functional properties.

Several different methods have been developed to extract rice protein; generally, rice flour is first defatted for protein extraction. Rice protein consists of four fractions with different solvent solubility: albumin (water-soluble), globulin (salt-soluble), glutelin (alkali-soluble), and prolamin (alcohol-soluble). Globulin (about 12%) and glutelin (about 80%) are the two major proteins, and albumin (about 5%) and prolamin (about 3%) are minor ones (Juliano 1994). Glutelin of rice is a protein of high molecular weight (6×10^4 B 6×10^5), also soluble in dilute acid and composed of subunits bound by disulfide linkages (Tecson and others 1971), while rice globulin is composed of low-molecular-weight (12 to 20×10^3) protein components (Cagampang and others 1976). The storage protein fractions can be sequentially extracted by water, salt, ethanol, and alkali buffer (Luthe 1984). Extraction conditions must be optimized for high protein recovery: 70% n-propanol was recommended for prolamin extraction by Sugimoto and others (1986) after they had tested various con-

centrations of aqueous methanol, ethanol, isopropanol, n-propanol, and tert-butanol. A 98% of extraction efficiency for glutelin was obtained at pH 11.8 (Tecson and others 1971). The study also showed that, below pH 10, the solubility of glutelin was drastically reduced (El-Sayed and others 1986). Wheat gluten solvents, urea, aluminum, lactate, and phenol:acetic-acid: water were previously shown to be poor extractants for rice glutelin (Palmiano and others 1968). Rice albumin, globulin, prolamin, and glutelin were extracted and characterized using gel electrophoresis and isoelectric focusing (Padhye and Salunkhe 1979; Zarins and Chrastil 1992; Chrastil and Zarins 1994). Each of these proteins was found to consist of a few components.

Understanding basic physicochemical and functional properties of a protein is very important for its application in food products. The information on protein thermal properties is useful for food processing strategies and heat processing design. Differential scanning calorimetry was used to determine thermal properties of rice globulin (Gorinstein and others 1996). A low denaturation temperature (60.1 °C) was detected for the rice globulin. Surface hydrophobicity of protein is often used to evaluate protein functionality (Nakai and Li-Chan 1993). During drying, milling, and storage, denaturation and changes of functionality of the proteins can take place that may influence overall rice quality. Therefore, finding a method to monitor and control denaturation of rice proteins will be very useful for the rice industry. Our research focused on the extraction of the different rice proteins and in obtaining information on thermal and hydrophobic properties of rice proteins and their components.

Materials and Methods

Materials

Rice flour from broken rice (long grain, RL 100) was provided by Riceland Foods (Stuttgart, Ark., U.S.A.). Analytical reagents were purchased from Fisher Scientific (Pittsburgh, Pa., U.S.A.) and Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Protein extraction

Rice flour (100 g) was defatted with 400 ml hexane (Figure

1). The defatted rice flour was dried under a hood at ambient temperature for 24 h. The flour was then extracted by shaking with 400 mL distilled water at 20 °C for 4 h (albumin extract) and centrifuged at $3000 \times g$ for 30 min. After water extraction, the flour was extracted with 400 mL of 5% NaCl at 20 °C for 4 h (globulin extract) and centrifuged at $3000 \times g$ for 30 min. The flour was then extracted for glutelin with 300 mL of 0.02 M NaOH (to pH 11.0), at 20 °C for 30 min, and followed by prolamin extraction with 300 mL of 70% ethanol at 20 °C for 4 h (Sugimoto and others 1986). Each extraction was repeated two times in order to remove all the protein of each fraction.

Separation of proteins from extracts

Albumin, globulin, and glutelin were precipitated from their supernatants by adjusting pH to their isoelectric points (Ips). The Ips were determined by subjecting a portion of each supernatant to a pH ranging from 3.0 to 10.0 and determining the turbidity (optical density at 320 nm) with a spectrophotometer (Varian Series-634; Varian Techtron, Pty. Ltd, Melbourne, Australia). The pH that gave the maximum turbidity was taken as the Ip. Prolamin was precipitated by adding acetone to the supernatant, according to Tecson and others (1971). The precipitated proteins (albumin, globulin, glutelin, and prolamin) were washed twice with distilled water, adjusted to pH 7.0, freeze-dried, and then stored at 4 °C.

Pure starch was prepared from the residue (crude starch) after protein extraction. The residue was sequentially washed with water (600 mL), ethanol (300 mL), acetone (300 mL), and diethyl ether (300 mL), then centrifuged ($1000 \times g$ for 10 min) and dried under a hood for 24 h to remove residual solvents.

Protein contents

Protein contents in defatted rice flour, supernatants, and freeze-dried protein products were determined by the Kjeldahl method (AACC 1983), and the nitrogen content was multiplied by 5.95 (Juliano 1994). Protein extraction efficiency (%) was calculated as a percentage of the summed amount of proteins in the four supernatants to the total protein of defatted rice flour. Recovered efficiency (%) of each protein was calculated as a percentage of the amount of pre-

Table 1—Protein contents of rice flour and recovery efficiency

	Protein content (%)	% of total protein	Recovered Efficiency (%)
Rice flour	8.75	-	-
Albumin	0.38	4.45	82.3
Globulin	1.12	13.11	91.5
Glutelin	6.81	79.74	93.2
Prolamin	0.21	2.46	90.0

cipitated, freeze-dried protein from supernatants to the total protein in the supernatants.

Differential scanning calorimetry (DSC)

The thermal characteristics of the four proteins (albumin, globulin, glutelin, and prolamin) as well as rice flour and rice starch were examined by a Perkin-Elmer (Norwalk, Conn., U.S.A.) DSC-4 which was equipped with a software program of thermal analysis data station (Pyris-1-DSC; Perkin-Elmer Corp., Norwalk, Conn., U.S.A.). About 10 mg of the fractionated proteins (albumin/globulin/glutelin/prolamin) was placed into a stainless steel pan (large-volume capsule) and accurately weighed, and 35 L of 0.01 M phosphate buffer (pH 7.5) was added. The samples were equilibrated at 4 °C overnight and scanned during temperature increases from 4 to 120 °C at 10 °C/min. Onset denaturation temperature, denaturation temperature, and enthalpy value of denaturation were computed from each thermogram by the software.

Surface hydrophobicity

The 1% globulin and 1% glutelin solutions were prepared by dissolving the proteins in 0.01 M phosphate buffer, respectively. The 1% glutelin solution was adjusted to pH 9.0 with 1 N NaOH. The two 1% protein solutions were then heated in a water bath at varying temperatures (45 to 95 °C) for 10 min. Protein solution samples were diluted to 0.00125, 0.0025, 0.005, 0.01, and 0.02% with 0.01 M phosphate buffer (Wu and others 1998). Ten L of 1-anilino-8-naphthalene sulfonate (ANS) solution (8 mM in 0.01 M phosphate buffer) was added to 4.0 mL of the protein solutions immediately before measurement. Fluorescence intensity of ANS-protein conjugates was measured with a Kontron Model SF23/B spectrofluorometer (Kontron Ltd, Zurich, Switzerland) at excitation and emission wavelengths of 390 and 470 nm, respectively (Wang and others 1999). The slope of the ANS fluorescence intensity against protein concentration (%) was calculated by linear regression and used as an index of the protein hydrophobicity.

Results and Discussion

Protein extraction and recovery efficiency

The rice flour (long grain, RL 100) contained 8.8% total protein (Table 1). Of the total protein in rice flour, 97.4% was extracted into supernatants by our methods (Figure 1). The total protein contained 4.5% albumin, 13.1% globulin, 79.7% glutelin, and 2.6% prolamin. These results are in agreement with the values reported by Juliano (1994).

Turbidity measurements showed that albumin and glutelin had maximum absorbance (optical density at 320 nm) at pH 4.1 and 4.8 (Figure 2), respectively, suggesting that these pH values were their Ips, at which the maximum amounts of protein could be precipitated from the protein supernatant. Two absorbance peaks (pH 4.3 and pH 7.9) were observed

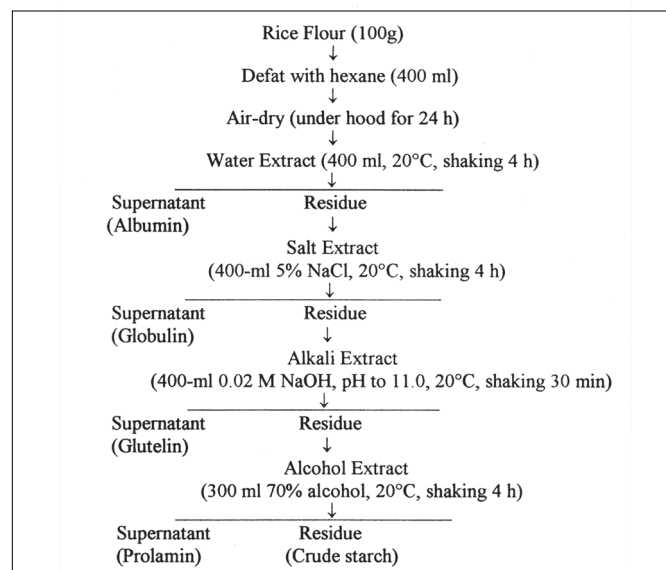


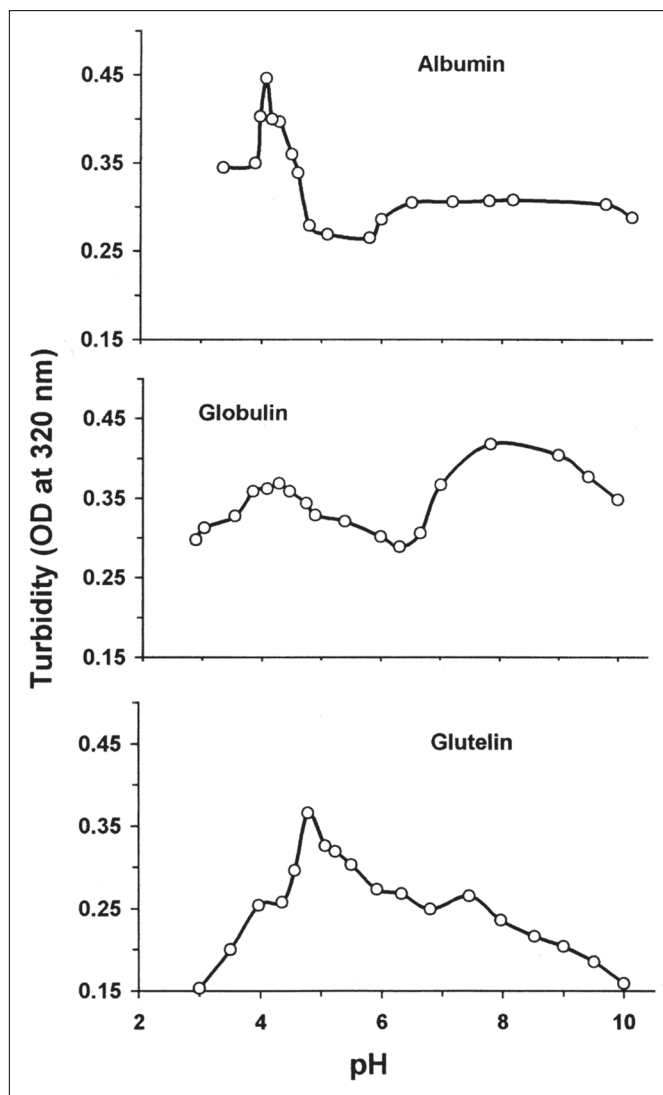
Figure 1—Flow diagram for extraction of rice proteins.

Table 2—Thermal properties of rice flour, starch and proteins

Samples	Denaturation temperature (°C)	Enthalpy value of denaturation (Jg)
Albumin	73.3 ^a	2.88 ^a
Globulin	78.9 ^b	3.14 ^b
Glutelin	82.2 ^c	3.79 ^c
Prolamin	NA	NA
Starch	84.7 ^e	10.53 ^e
Rice flour	80.5 ^d	8.49

a,b,c,d,e Means within a column followed by the same superscript letter is not significantly different ($P < 0.05$)

for rice globulin (Figure 2), suggesting that this protein had two Ips. Increasing pH values in acid pH range (pH 3 to 6.3) or alkali pH range (pH 6.7 to 10) first increased turbidity and then decreased turbidity. Adjusting the pH (3.0 to 11.0) of 70% ethanol extract did not result in aggregation of prolamin, and turbidity did not change with an increase or a decrease of pH. This protein was separated by adding acetone (Tecson and others 1971).

**Figure 2—Turbidity changes of rice protein (albumin, globulin and glutelin) with pH values (3.0-10.0).**

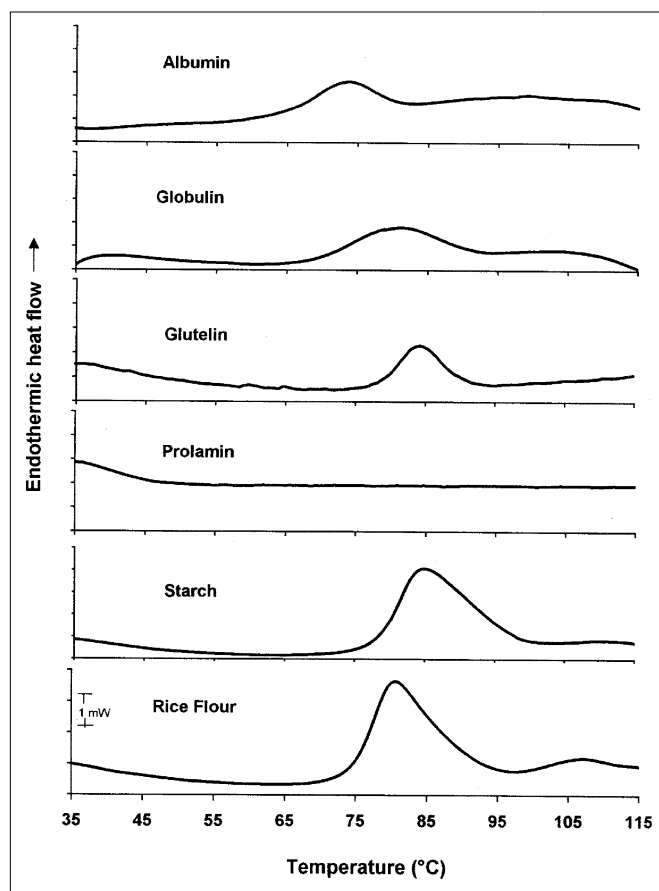
The rice flour (long grain, 100) contained 8.75% protein. This protein contained 4.5% water-soluble albumin, 13.1% salt-soluble globulin, 79.7% alkali-soluble glutelin, and 2.6% alcohol-soluble prolamin. A total of 97.4% protein was extracted from rice flour. At the Ips of rice proteins (albumin:pH 4.1; globulin:pH 4.3; glutelin:pH 4.8), 82.3% albumin, 91.5% globulin, and 93.2% glutelin were recovered from the respective supernatants (Table 1). Four freeze-dried protein products contained a high percentage of protein: albumin, 85.0%; globulin, 88.5%; glutelin, 94.3% and prolamin, 92.8%. These protein products were used to investigate protein components, denaturation, and surface hydrophobicity.

Thermal properties

Thermal analysis of proteins, rice flour, and rice starch can provide information on their cooking quality properties and also provide information on their physical and chemical energetic effects, including denaturation, crystallization, gelatinizing, glass transition, or other reactions.

Thermographs of the four rice proteins, rice starch, and their parent rice flour upon heat treatment (20 to 120 °C) are shown in Figure 3. A single enthalpy peak was detected for albumin, globulin, glutelin, starch, and rice flour. No peak was detected for prolamin.

The denaturation temperatures of albumin, globulin, and glutelin were 73.3, 78.9, and 82.2 °C, respectively (Table 2). A denaturation temperature at 60.1 °C was reported previously for rice globulin (Gorinstein and others 1996), which was

**Figure 3—Thermographs of rice proteins (albumin, globulin, glutelin and prolamin), rice starch and rice flour.**

18.8 °C lower than this determination (78.9 °C) in Table 2. Gorinstein and others (1996) in the same publication also reported 20 °C lower denaturation temperature (56 °C) for soybean globulin than others had determined (Nagano and others 1994). Gelatinizing temperature of rice starch was 84.7 °C (Table 2). Rice flour, mainly containing 8.8% protein and about 80% starch, had an intermediate transition temperature (80.5 °C) between proteins and starch. This peak temperature may reflect the interaction of rice proteins and starch. These results indicate that heating at 73 °C to 85 °C is critical in rice cooking quality and the formation of textural structure.

Enthalpy values of albumin (2.88 J/g) and globulin (3.14 J/g) were also lower than that of glutelin (3.79 J/g; Table 2), suggesting that these two proteins can more easily be denatured. The enthalpy value of starch (10.53 J/g) was much higher than that of proteins. Again, rice flour had an intermediate enthalpy value (8.49 J/g) between those of proteins and starch. No denaturation peak was detected for prolamin, probably due to denaturation by alcohol treatment during extraction.

Protein denaturation and surface hydrophobicity

In general, protein denaturation results in an increase of hydrophobicity, due to exposure of hydrophobic groups that are folded inside the intact native protein molecule (Mine 1997). Establishing the relationship of rice protein denaturation to hydrophobicity will help to control rice quality by providing a method to detect rice protein denaturation during drying, milling, storage, and process of rice.

Figure 4 shows changes of surface hydrophobicity of the two major rice proteins (globulin and glutelin) with heating (45 to 95 °C). The surface hydrophobicity of globulin progressively increased upon heat treatment from 45 °C to 80 °C for 10 min and leveled off from 80 °C to 95 °C for 10 min (Figure 4). Heat treatment at 80 °C for 10 min probably fully unfolded or denatured all globulin molecules and thus further heat treatment (> 80 °C) did not result in a change in surface hydrophobicity.

Heating glutelin at a low temperature (45 to 60 °C) did not significantly increase its surface hydrophobicity, and the treatment at high temperatures (65 to 95 °C) significantly increased surface hydrophobicity in a nearly linear manner ($y = 6.95 \times -229.02$; $R^2 = 0.96$). Surface hydrophobicity (234.8/slope index) of native globulin (without heat treatment) was

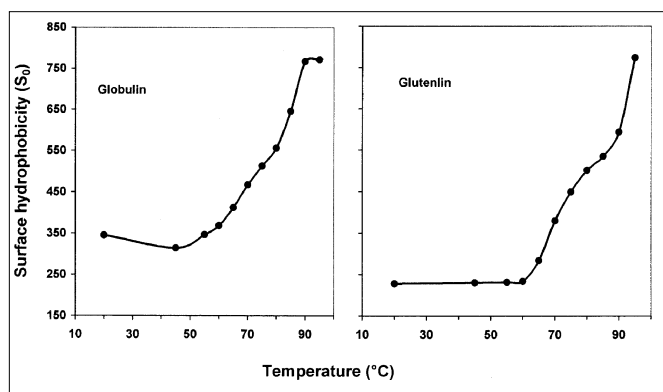


Figure 4—Effects of heat treatment at several temperatures (45–95 °C) on surface hydrophobicity of rice globulin and glutelin.

higher than that of native glutelin (189.3). The higher temperature led to a higher degree of protein denaturation (Ju and others 1997; Ju and others 1999). The higher degree of denatured proteins showed higher surface hydrophobicity (Figure 4). This result suggests that surface hydrophobicity may be used to evaluate the extent of protein denaturation in rice during drying, milling, storage and processing.

Conclusions

RICE FLOUR PROTEINS INCLUDING ALBUMIN, GLOBULIN, GLUTELIN, and prolamin can be effectively extracted by using appropriate solvents (water/salt/alkali/alcohol solutions). The denaturation temperature and enthalpy value of these proteins, except prolamin, can be detected using DSC. Heat-denaturation of globulin and glutelin resulted in significant increases in their surface hydrophobicity. Surface hydrophobicity of the proteins may be used to monitor protein denaturation during rice drying, milling, storage, and processing.

References

- AACC 1983. Approved methods of the AACC. Methods 22B60, 8th ed. St. Paul, Minn.:Amer Assoc of Cereal Chemists. NUMBER OF PAGES???
- Bean MM, Nishita KD. 1985. Rice flours for baking. In Juliano BO, editor. Rice: chemistry and technology, 2nd ed. St. Paul: Amer Assoc of Cereal Chemists. P 539-556.
- Cagampang GB, Perdon AA, Juliano BO. 1976. Changes in salt-soluble proteins of rice during rain development. *Biochem* 15(10):1425-1429.
- Chrastil J. 1992. Correlation between the physicochemical and functional properties of rice. *J Agric Food Chem* 40(6):1683-1686.
- Chrastil J, Zarins Z. 1994. Changes in peptide subunit composition of albumins, globulins, prolamins, and oryzenin in maturing rice grains. *J Agric Food Chem* 42(10):2152-2155.
- El-Sayed MA, Youssef MM, Adel-Shehata A, El-Mahdy AR. 1986. Extractability and functionality of rice proteins and their application as meat extenders. *Food Chem* 20(1):79-83.
- Gorinstein S, Zemser M, Paredes LO. 1996. Structural stability of globulins. *J Agric Food Chem* 44(1):100-105.
- Ju ZY, Hettiarachchy NS, Kilara A. 1999. Thermal properties of whey protein aggregates. *J Dairy Sci* 82(9):1882-1889.
- Ju ZY, Otte J, Zakora M, Qvist KB. 1997. Enzyme-induced gelation of whey proteins: Effect of protein denaturation. *Int Dairy J* 7(1):71-78.
- Juliano BO. 1994. Polysaccharides, proteins, and lipids of rice. In: Rice: Chemistry and Technology. St. Paul, Minn.:Amer Assoc of Cereal Chemists. P 98-141.
- Luthe DS. 1984. Storage protein accumulation in developing rice (*Oryza sativa* L.) seeds. *Plant Sci Lett. Limerick, Ire.:Elsevier*. 32(1):147-158.
- Marshall WG, Wordsworth JL. 1994. Rice Science and Technology. New York:Marcel Dekker, Inc. 237-259.
- Mine Y. 1997. Effect of dry heat and mild alkaline treatment on functional properties of egg white proteins. *J Agric Food Chem* 45(8):2924-2928.
- Nagano T, Mori H, Nishinari K. 1994. Effect of heating and cooling on the gelation kinetics of 7S globulin from soybeans. *J Agric Food Chem* 42(7):1415-1419.
- Nakai S, Li-Chan E. 1993. Recent advances in structure and function of food protein: QSAR approach. *Crit Rev in Food Sci and Nutr* 33(2):477-499.
- Padhye VW, Salunkhe DK. 1979. Extraction and characterization of rice proteins. *Cereal Chem* 106(3):389-393.
- Palmiano EP, Almazan, AM, Juliano BO. 1968. Physiochemical properties of protein of developing and mature rice grain. *Cereal Chem* 45(1):1-6.
- Sugimoto T, Tanaka K, Kasai Z. 1986. Improved extraction of rice prolamin. *Agric Biol Chem Tokyo. Agril Chem Soc of Japan* 50(11):2409-2411.
- Tecson EMS, Esmama BV, Lontok LP, Juliano BO. 1971. Studies on the extraction and composition of rice endosperm, glutelin, and prolamin. *Cereal Chem* 48(2):168-181.
- Wang M, Hettiarachchy NS, Burks W, Siebenmorgen T. 1999. Preparation and functional properties of protein isolates from nonheat-treated rice bran. *J Agric Food Chem* 47(2):411-416.
- Wu W, Hettiarachchy NS, Qi M. 1998. Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. *J Amer Oil Chemists Soc* 75(7):845-850.
- Zarins Z, Chrastil J. 1992. Separation and purification of rice oryzenin subunits by anion-exchange and gel-permeation chromatography. *J Agric Food Chem* 40(9):1599-1601.

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